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(21) International Application Number: PCT/GB94/00495 (22) International Filing Date: 14 March 1994 (14.03.94) (30) Priority Data: 9305348.6 16 March 1993 (16.03.93) GB 9320360.2 2 October 1993 (02.10.93) GB (71) Applicant (for all designated States except US): BRITISH BIO-TECHNOLOGY LIMITED [GB/GB]; Watlington Road, Cowley, Oxford OX4 5LY (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): CRIMMIN, Michael, John [GB/GB]; British Bio-technology Limited, Watlington Road, Cowley, Oxford OX4 5LY (GB). BECKETT, Paul, Raymond [GB/GB]; British Bio-technology Limited, Watlington Road, Cowley, Oxford OX4 5LY (GB). DAVIS, Mark, Hampton [GB/GB]; British Bio-technology Limited, Watlington Road, Cowley, Oxford OX4 5LY (GB). (74) Agent: WALLS, Alan, J.; British Bio-technology Limited, Watlington Road, Cowley, Oxford OX4 5LY (GB).		(81) Designated States: AU, BR, CA, CN, CZ, DE, FI, GB, HU, JP, KR, NO, NZ, RU, UA, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: HYDROXAMIC ACID DERIVATIVES AS METALLOPROTEINASE INHIBITORS (57) Abstract <p>Compounds of formula (I), wherein R₂ represents a C₂-C₆ alkyl group which may contain an ether or thioether linkage; R₃ represents (a) the side chain of a naturally occurring alpha-amino acid in which any carboxylic acid group may be esterified or amidated, any hydroxyl or thiol group may be acylated or alkylated (etherified) and any amino group may be acylated, or (b) a group R₆(A)_n wherein n is 0 or 1, A represents a divalent C₁-C₆ alkyl or C₂-C₆ alkenyl group optionally interrupted by one or more -O-, or -S- atoms or -N(R₇)- groups where R₇ is hydrogen or C₁-C₆ alkyl, and R₆ is a phenyl or heterocyclyl group either of which may be substituted, or (except where n is 0) a hydrogen atom; R₄ represents hydrogen or methyl; R₅ represents hydrogen, C₁-C₆ alkyl or phenyl(C₁-C₆ alkyl), and salts, solvates and hydrates thereof, are inhibitors of metalloproteinases involved in tissue degradation.</p> <div style="text-align: center;"> <p style="text-align: right;">(I)</p> </div>		

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Hydroxamic Acid Derivatives as Metalloproteinase Inhibitors

The present invention relates to therapeutically active hydroxamic acid derivatives, to processes for their preparation, to pharmaceutical compositions containing them, and to the use of such compounds in medicine. In particular, the compounds are inhibitors of metalloproteinases involved in tissue degradation, and are in addition inhibitors of the release of tumour necrosis factor from cells

Background to the Invention

There is evidence that compounds which have the property of inhibiting the action of metalloproteinases involved in connective tissue breakdown such as collagenase, stromelysin and gelatinase (known as "matrix metalloproteinases", and herein referred to as MMPs) are potentially useful for the treatment or prophylaxis of conditions involving such tissue breakdown, for example rheumatoid arthritis, osteoarthritis, osteopenias such as osteoporosis, periodontitis, gingivitis, corneal, epidermal or gastric ulceration, and tumour metastasis, invasion, and growth.

Metalloproteinases are characterised by the presence in the structure of a zinc(II) ionic site. It is now known that there exists a range of metalloproteinase enzymes that includes fibroblast collagenase (Type 1), PMN-collagenase, 72 kDa-gelatinase, 92 kDa-gelatinase, stromelysin, stromelysin-2 and PUMP-1 (J.F. Woessner, FASEB J, 1991, 5, 2145-2154). Many known MMP inhibitors are peptide derivatives, based on naturally occurring amino acids, and are analogues of the cleavage site in the collagen molecule. A recent paper by Chapman et al J. Med. Chem. 1993, 36, 4293-4301 reports some general structure/activity findings in a series of N-carboxyalkyl peptides. Other known MMP inhibitors are less peptidic in structure, and may more properly be viewed as pseudopeptides or peptide mimetics. Such compounds usually have a functional group capable of binding to the zinc (II) site in the MMP, and known classes include those in which the zinc binding group is a hydroxamic acid, carboxylic acid, sulphydryl, and oxygenated phosphorus (eg phosphinic acid and phosphonic acid) groups.

The following patent publications published prior to the first claimed priority date of this application disclose hydroxamic acid-based MMP inhibitors:

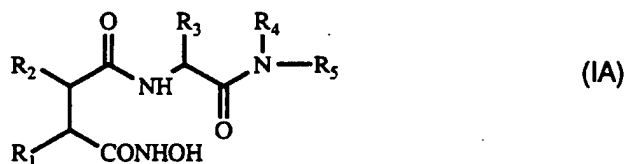
US 4599361	(Searle)
EP-A-0231081	(ICI)
EP-A-0236872	(Roche)
EP-A-0274453	(Bellon)
WO 90/05716	(British Bio-technology Ltd ("BBL"))
WO 90/05719	(BBL)
WO 91/02716	(BBL)
WO 92/09563	(Glycomed)
EP-A-0497192	(Roche)
WO 92/13831	(BBL)
EP-A-0489577	(Celltech)
EP-A-0489579	(Celltech)
WO 92/22523	(Research Corporation Technologies)

The following patent publications published after the first claimed priority date of this application, but before its filing date, also relate to hydroxamic acid-based MMP inhibitors:

US 6256657	(Sterling Winthrop)
WO 93/09090	(Yamanouchi)
WO 93/09097	(Sankyo)
WO 93/20047	(BBL)
WO 93/21942	(BBL)
WO 93/24449	(Celltech)
WO 93/24475	(Celltech)
EP-A-0574758	(Roche)
WO 94/02446	(BBL)
WO 94/02447	(BBL)

The intrinsic potency of compounds within the broad structural groups of hydroxamic derivatives disclosed in the above publications against particular MMPs can be high. For example, many have a collagenase IC₅₀ by the *in vitro* test method of Cawston and Barrett, (Anal. Biochem., 99, 340-345, 1979) of less than 50 nM. Unfortunately, however, the pharmacokinetic properties of the specific compounds disclosed in those publications have generally been disappointing. Identifying hydroxamic acid-based MMP inhibitors having a good balance of high intrinsic activity and good pharmacokinetic properties, for example prolonged effective plasma concentrations following oral dosing such that the compounds have high *in vivo* activity in target diseases or conditions such as rheumatoid or osteoarthritis, or cancer, remains a much sought after goal in the art.

With a few exceptions, the hydroxamic acid derivatives disclosed in the above publications can be regarded as having the following basic structure (IA):



wherein the five substituents R₁ - R₅ may vary according to the detailed disclosure of each publication. The balance of intrinsic level of activity, degree of specificity of activity for a particular category of MMP, and pharmacokinetic properties can vary in an unpredictable way as the substituents R₁ - R₅ are varied.

Tumour necrosis factor (herein referred to as "TNF") is a cytokine which is produced initially as a cell-associated 28kD precursor. It is released as an active, 17kD form, which can mediate a large number of deleterious effects in vivo. When administered to animals or humans it causes inflammation, fever, cardiovascular effects, haemorrhage, coagulation and acute phase responses, similar to those seen during acute infections and shock states. Chronic administration can also

cause cachexia and anorexia. Accumulation of excessive TNF can be lethal.

There is considerable evidence from animal model studies that blocking the effects of TNF with specific antibodies can be beneficial in acute infections, shock states, graft versus host reactions and autoimmune disease. TNF is also an autocrine growth factor for some myelomas and lymphomas and can act to inhibit normal haematopoiesis in patients with these tumours.

Compounds which inhibit the production or action of TNF are therefore thought to be potentially useful for the treatment or prophylaxis of many inflammatory, infectious, immunological or malignant diseases. These include, but are not restricted to, septic shock, haemodynamic shock and sepsis syndrome, post ischaemic reperfusion injury, malaria, Crohn's disease, mycobacterial infection, meningitis, psoriasis, congestive heart failure, fibrotic disease, cachexia, graft rejection, cancer, autoimmune disease, rheumatoid arthritis, multiple sclerosis, radiation damage, toxicity following administration of immunosuppressive monoclonal antibodies such as OKT3 or CAMPATH-1 and hyperoxic alveolar injury.

Since excessive TNF production has been noted in several diseases or conditions also characterised by MMP-mediated tissue degradation, compounds which inhibit both MMPs and TNF production may have particular advantages in the treatment or prophylaxis of diseases or conditions in which both mechanisms are involved.

Recently, WO 93/20047 disclosed a class of hydroxamic acid based MMP inhibitors which also are active in inhibiting TNF production.

Brief Description of the Invention

This invention is based on the discovery of a novel group of compounds of general formula (IA), principally characterised in that R_1 is an allyl group ($\text{CH}_2=\text{CH}-\text{CH}_2-$), which have a good balance of high intrinsic activity and good pharmacokinetic properties. In particular the compounds produce prolonged effective plasma

concentrations following oral dosing (as opposed to a high initial peak concentration followed by relatively rapid exponential decline). The compounds of the invention also have activity as inhibitors of the production of TNF. Such properties imply that the compounds of the invention find particular application in the treatment of diseases and conditions which benefit from prolonged exposure to effective inhibitory concentrations of MMP and/or TNF inhibitors, such as rheumatoid or osteoarthritis, and cancer.

Of the patent publications listed above, only the following claim compounds of formula (IA) in which R_1 is an alkenyl group:

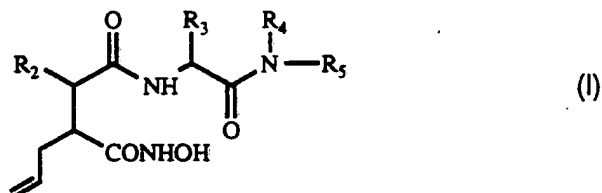
WO 90/05716	(BBL)
WO 91/02716	(BBL)
EP-A-0489577	(Celltech)
EP-A-0489579	(Celltech)
WO 93/24449	(Celltech)
WO 93/24475	(Celltech)

However, none discloses any specific example of a compound (IA) in which R_1 is alkenyl, or the properties expected to be possessed by such compounds.

Specifically, none discloses any specific example of a compound (IA) in which R_1 is alkenyl. In fact, in all of the compounds of formula (IA) specifically disclosed in EP-A-0489577, EP-A-0489579, WO 93/24449, and WO 93/24475, R_1 is hydrogen, while in compounds of formula (IA) disclosed in WO 90/05716 and WO 91/02716 R_1 is hydrogen or methyl (or in one instance phenylsulphanylmethyl). Therefore, insofar as the prior art can be regarded as effectively disclosing compounds (IA) in which R_1 is alkenyl, it treats R_1 = alkenyl as equivalent to R_1 = hydrogen or methyl (and phenylsulphanylmethyl), and has not recognised the activity/bioavailability benefits found by the present inventors to be associated with R_1 = allyl, the main characterising feature of compounds of the present invention.

Detailed Description of the Invention

According to one aspect of the present invention, there is provided a compound of formula (I) :



wherein

R_2 represents a C_2 - C_6 alkyl group which may contain an ether or thioether linkage;

R_3 represents (a) the side chain of a naturally occurring alpha-amino acid in which any carboxylic acid groups may be esterified or amidated, any hydroxyl or thiol groups may be acylated or alkylated (etherified) and any amino groups may be acylated, or (b) a group $R_6(A)_n$ wherein n is 0 or 1, A represents a divalent C_1 - C_6 alkyl or C_2 - C_6 alkenyl group optionally interrupted by one or more $-O-$, or $-S-$ atoms or $-N(R_7)-$ groups where R_7 is hydrogen or C_1 - C_6 alkyl, and R_6 is a phenyl or heterocyclyl group either of which may be substituted, or (except where n is 0) a hydrogen atom;

R_4 represents hydrogen or methyl;

R_5 represents hydrogen, C_1 - C_6 alkyl or phenyl(C_1 - C_6 alkyl),

or a salt, solvate or hydrate thereof.

As used herein the term " C_1 - C_6 alkyl" refers to a straight or branched chain alkyl moiety having from 1 to 6 carbon atoms, including for example, methyl, ethyl, propyl, isopropyl, butyl, *t*-butyl, pentyl and hexyl. " C_2 - C_6 alkyl" of course means straight or branched chain alkyl moiety having from 2 to 6 carbon atoms, and examples include the foregoing, except methyl.

The term " C_2 - C_6 alkyl group which may contain an ether or thioether linkage" means a group of formula $Z-X-Y$ wherein Z is C_2 - C_6 alkyl, X is $-O-$ or $-S-$, Y is a

bond or C₂-C₆ alkyl, provided that the total number of C atoms does not exceed 5. Examples of such groups include CH₃(CH₂)₂OCH₂-, and CH₃(CH₂)₂S-.

The term "C₂-C₆ alkenyl" refers to a straight or branched chain alkenyl moiety having from 2 to 6 carbon atoms and having in addition one double bond of either E or Z stereochemistry where applicable. This term would include, for example, vinyl, 1-propenyl, 1- and 2-butenyl and 2-methyl-2-propenyl.

As used herein, the term "side chain of a naturally occurring alpha-amino acid" includes the side chains of alanine, arginine, asparagine, aspartic acid, cysteine, cystine, glutamic acid, glycine, histidine, 5-hydroxylysine, 4-hydroxyproline, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, alpha-aminoadipic acid, alpha-amino-n-butyric acid, 3,4-dihydroxyphenylalanine, homoserine, alpha-methylserine, ornithine, pipecolic acid, and thyroxine. The amino acid side chains may be protected; for example the carboxyl groups of aspartic acid, glutamic acid and alpha-aminoadipic acid may be esterified (for example as a C₁-C₆ alkyl ester), the amino groups of lysine, ornithine, 5-hydroxylysine, 4-hydroxyproline may be converted to amides (for example as a COC₁-C₆ alkyl amide) or carbamates (for example as a C(=O)OC₁-C₆ alkyl or C(=O)OCH₂Ph carbamate), the hydroxyl groups of 5-hydroxylysine, 4-hydroxyproline, serine, threonine, tyrosine, 3,4-dihydroxyphenylalanine, homoserine, alpha-methylserine and thyroxine may be converted to ethers (for example a C₁-C₆ alkyl or a (C₁-C₆ alkyl)phenyl ether) or esters (for example a C(=O)C₁-C₆ alkyl ester) and the thiol group of cysteine may be converted to thioethers (for example a C₁-C₆ alkyl thioether) or thioesters (for example a C(=O)C₁-C₆ alkyl thioester).

The unqualified term "heterocyclyl" refers to a 5-7 membered heterocyclic ring containing one or more heteroatoms selected from S, N and O, and optionally fused to a benzene ring, including for example, pyrrolyl, furyl, thienyl, imidazolyl, oxazolyl, oxadiazolyl, thiazolyl, pyrazolyl, isoxazolyl, triazolyl, thiadiazolyl,

pyridazinyl, pyrazinyl, triazinyl, pyridinyl, pyrrolidinyl, pyrimidinyl, morpholinyl, piperizinyl, indolyl, benzimidazole, phthalimido, 1,2-dimethyl-3,5-dioxo-1,2,4-triazolidin-4-yl, 3-methyl-2,5-dioxo-1-imidazolidinyl and 3,4,4-trimethyl-2,5-dioxo-1-imidazolidinyl.

Salts of the compounds of the invention include physiologically acceptable acid addition salts for example hydrochlorides, hydrobromides, sulphates, methane sulphonates, p-toluenesulphonates, phosphates, acetates, citrates, succinates, lactates, tartrates, fumarates and maleates. Salts may also be formed with bases, for example sodium, potassium, magnesium, and calcium salts.

Unless otherwise specified in the context in which it occurs, the term "substituted" as applied to any moiety herein means substituted with up to four substituents, each of which independently may be C₁-C₆ alkoxy, hydroxy, thio, C₁-C₆ alkylthio, amino, halo (including fluoro, chloro, bromo and iodo), trifluoromethyl, nitro, -COOH, -CONH₂ or -CONHRA wherein RA is a C₁-C₆ alkyl group or the residue of a natural alpha-amino acid.

There are several chiral centres in the compounds according to the invention because of the presence of asymmetric carbon atoms. The presence of several asymmetric carbon atoms gives rise to a number of diastereomers with R or S stereochemistry at each chiral centre. General formula (I), and (unless specified otherwise) all other formulae in this specification are to be understood to include all such stereoisomers and mixtures (for example racemic mixtures) thereof.

In the compounds of the invention, the preferred stereochemistry is in general as follows:

C atom carrying the allyl group and hydroxamic acid moiety - S,

C atom carrying the R₂ group - R,

C atom carrying the R₃ group - S,

but mixtures in which the above configurations predominate are also contemplated.

Presently preferred compounds include those in which, independently or in any combination:

R₂ represents an isobutyl group;

R₃ represents a phenylmethyl (benzyl) group in which the phenyl ring is optionally substituted with a C₁-C₄ alkoxy or HOOC-(C₁-C₄ alkyl)-O- group, a thienylmethyl group, a C₁-C₆ alkyl group for example a tertbutyl or isobutyl group, or a CH₂CO₂ (C₁-C₄)alkyl or CH₂CH₂CO₂ (C₁-C₄)alkyl group;

R₄ represents a hydrogen atom;

R₅ represents a (C₁-C₄)alkyl group or a benzyl group;

Interesting compounds of the invention are:

3R-(3-methoxycarbonyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-2S-2-propenyl-hexanohydroxamic acid;

3R-(1S-methylcarbamoyl-2-thien-2-yl-ethylcarbamoyl)-5-methyl-2S-2-propenyl-hexanohydroxamic acid;

3R-(3-Methyl-1S-methylcarbamoyl-butylcarbamoyl)-5-methyl-2S-2-propenyl-hexanohydroxamic acid;

2S-[1S-methylcarbamoyl-2-oxadiazol-5-yl-ethylcarbamoyl)-5-methyl-2S-2-propenyl-hexanohydroxamic acid;

and salts, solvates or hydrates thereof.

A compound of the invention which is preferred for its high intrinsic activity, and for its good pharmacokinetic properties, evidenced for example by its high *in vivo* activity following oral administration in a standard rat adjuvant arthritis model, is:

3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-2S-2-propenyl-hexanohydroxamic acid and its salts, solvates or hydrates.

A further compound of the invention which is especially preferred for its high

intrinsic activity, and for its good pharmacokinetic properties, evidenced for example by its prolonged high plasma concentrations following oral administration, is:

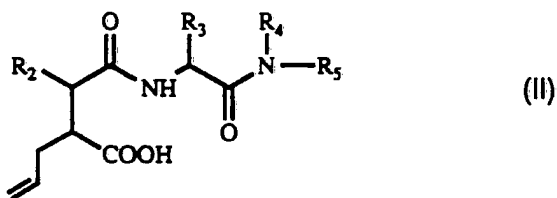
3R-(2,2-dimethyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-2S-2-propenyl-hexanohydroxamic acid and its salts, solvates or hydrates.

Another compound of the invention which is preferred for its good intrinsic activity in inhibiting especially stromelysin, is:

3R-(2-phenyl-1S-phenylmethylcarbamoyl-ethylcarbamoyl)-5-methyl-2S-2-propenyl-hexanohydroxamic acid and its salts, solvates or hydrates.

Compounds according to the present invention may be prepared by methods known *per se* in the art, and by the following process, which forms another aspect of the invention, namely a process for the preparation of a compound of formula (I) comprising:

(a) coupling an acid of general formula (II)



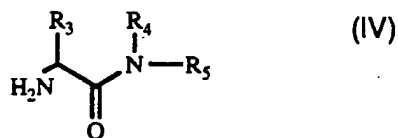
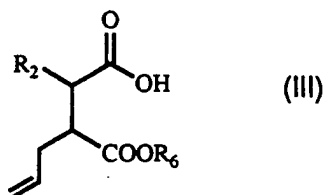
or an activated derivative thereof with hydroxylamine, O-protected hydroxylamine, or a salt thereof, R_2 , R_3 , R_4 , and R_5 being as defined in general formula (I) except that any substituents in R_2 , R_3 , R_4 , and R_5 which are potentially reactive with hydroxylamine, O-protected hydroxylamine or their salts may themselves be protected from such reaction, then removing any protecting groups from the resultant hydroxamic acid moiety and from any protected substituents in R_2 , R_3 , R_4 , and R_5 , and

- (b) optionally converting a compound of general formula (I) into another compound of general formula (I).

Conversion of (II) to an activated intermediate such as the pentafluorophenyl, hydroxysuccinyl, or hydroxybenztriazyl ester may be effected by reaction with the appropriate alcohol in the presence of a dehydrating agent such as dicyclohexyl carbodiimide (DCC), N,N-dimethylaminopropyl-N'-ethyl carbodiimide (EDC), or 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ).

Protecting groups as referred to above are well known per se, for example from the techniques of peptide chemistry. Amino groups are often protectable by benzyloxycarbonyl, t-butoxycarbonyl or acetyl groups, or in the form of a phthalimido group. Hydroxy groups are often protectable as readily cleavable ethers such as the t-butyl or benzyl ether, or as readily cleavable esters such as the acetate. Carboxy groups are often protectable as readily cleavable esters, such as the t-butyl or benzyl ester.

A compound of general formula (II) can be prepared by coupling an acid of formula (III) or an activated derivative thereof with an amine of formula (IV)



wherein R_2 , R_3 , R_4 , and R_5 are as defined in general formula (I) and R_6 represents C_1 - C_6 alkyl (eg t-butyl), or benzyl. Active derivatives of acids (III) include activated esters such as the pentafluorophenyl ester, acid anhydrides and acid halides, eg chlorides.

As mentioned above, compounds of formula (I) are useful in human or veterinary medicine since they are active as inhibitors of MMPs. A further advantage of the compounds of formula (I) lies in their ability to inhibit the release of tumour necrosis factor (TNF) from cells.

TNF is a cytokine which is produced initially as a cell-associated 28kD precursor, is released as an active, 17kD form (Jue, D-M et al., (1990) *Biochemistry* 29:8371-8377), which can mediate a large number of deleterious effects *in vivo*. When administered to animals or humans it causes inflammation, fever, cardiovascular effects, haemorrhage, coagulation and acute phase responses, similar to those seen during acute infections and shock states. Chronic administration can also cause cachexia and anorexia. Accumulation of excessive TNF can be lethal. There is considerable evidence from animal model studies that blocking the effects of TNF with specific antibodies can be beneficial in acute infections, shock states, graft versus host reactions and autoimmune disease. TNF is also an autocrine growth factor for some myelomas and lymphomas and can act to inhibit normal haematopoiesis in patients with these tumours.

Preventing the production or action of TNF is, therefore, predicted to be a potent therapeutic strategy for many inflammatory, infectious, immunological or malignant diseases. These include, but are not restricted to, septic shock, haemodynamic shock and sepsis syndrome (Mathison *et al.* (1988) *J. Clin. Invest.* 81:1925-1937; Miethke *et al.* (1992) *J. Exp. Med.* 175:91-98), post ischaemic reperfusion injury, malaria (Grau *et al.*, (1989) *Immunol. Rev.* 112:49-70); mycobacterial infection (Barnes *et al.* (1992) *Infect. Imm.* 60:1441-6), meningitis, psoriasis, congestive heart failure, fibrotic disease, cachexia, graft rejection, cancer, autoimmune disease, rheumatoid arthritis, multiple sclerosis, radiation damage, toxicity following administration of immunosuppressive monoclonal antibodies such as OKT3 or CAMPATH-1 and hyperoxic alveolar injury.

It has recently been shown that the effects of TNF are mediated by two peptides, TNF α and TNF β . Although these peptides have only 30% homology with each

other, they activate the same receptors and are encoded by immediately adjacent genes. As used herein, the term tumour necrosis factor or TNF therefore means tumour necrosis factor α and peptides having a high degree of sequence homology with, or substantially similar physiological effects to, TNF α , for example TNF β .

Accordingly in another aspect, this invention concerns:

- (i) a method of management (by which is meant treatment or prophylaxis) of diseases or conditions mediated by MMPs and/or TNF in mammals, in particular in humans, which method comprises administering to the mammal an effective amount of a compound of formula (I) above, or a pharmaceutically acceptable salt thereof; and
- (ii) a compound of formula (I) for use in human or veterinary medicine, particularly in the management (by which is meant treatment or prophylaxis) of diseases or conditions mediated by MMPs and/or TNF; and
- (iii) the use of a compound of formula (I) in the preparation of an agent for the management (by which is meant treatment or prophylaxis) of diseases or conditions mediated by MMPs and/or TNF.

Diseases or conditions mediated by MMPs include those involving tissue breakdown such as bone resorption, inflammatory diseases, dermatological conditions and tumour invasion by secondary metastases, in particular rheumatoid arthritis, osteoarthritis, periodontitis, gingivitis, corneal ulceration and tumour invasion by secondary metastases. Diseases or conditions mediated by TNF include inflammation, fever, cardiovascular effects, haemorrhage, coagulation and acute phase response, cachexia and anorexia, acute infections, shock states, graft versus host reactions and autoimmune disease.

In a further aspect of the invention there is provided a pharmaceutical or veterinary

composition comprising a compound of formula (I) together with a pharmaceutically or veterinarily acceptable excipient or carrier.

One or more compounds of general formula (I) may be present in the composition together with one or more excipient or carrier.

The compounds with which the invention is concerned may be prepared for administration by any route consistent with their pharmacokinetic properties. The compositions may be in the form of tablets, capsules, powders, granules, lozenges, liquid or gel preparations, such as oral, topical, or sterile parenteral solutions or suspensions. Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrrolidone; fillers for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tableting lubricant, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants for example potato starch, or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, glucose syrup, gelatin hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid, and if desired conventional flavouring or colouring agents.

The dosage unit involved in oral administration may contain from about 1 to 250mg, preferably from about 25 to 250mg of a compound of general formula I. A suitable daily dose for a mammal may vary widely depending on the condition of

the patient. However, a dose of a compound of general formula I of about 0.1 to 300mg/kg body weight, particularly from about 1 to 100mg/kg body weight may be appropriate.

For topical application to the skin, the drug may be made up into a cream, lotion or ointment. Cream or ointment formulations which may be used for the drug are conventional formulations well known in the art, for example as described in standard textbooks of pharmaceuticals such as the British Pharmacopoeia.

For topical application to the eye, the drug may be made up into a solution or suspension in a suitable sterile aqueous or non aqueous vehicle. Additives, for instance buffers such as sodium metabisulphite or disodium edeate; preservatives including bactericidal and fungicidal agents such as phenyl mercuric acetate or nitrate, benzalkonium chloride or chlorhexidine, and thickening agents such as hypromellose may also be included.

The dosage for topical administration will of course depend on the size of the area being treated. For the eyes, each dose may typically be in the range from 10 to 100mg of the drug.

The active ingredient may also be administered parenterally in a sterile medium. Depending on the vehicle and concentration used, the drug can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as a local anaesthetic, preservative and buffering agents can be dissolved in the vehicle.

For use in the treatment of rheumatoid arthritis, the drug can be administered by the oral route or by injection intra-articularly into the affected joint. The daily dosage for a 70kg mammal may be in the range 10mgs to 1gram.

The examples which follow serve to illustrate the invention but are not intended to limit the scope in any way. The amino acids used in the examples were

commercially available or were prepared according to literature procedures.

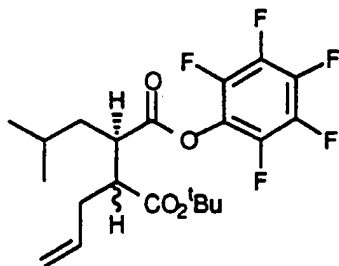
The following abbreviations have been used throughout:

DIPE	Diisopropyl ether
DMF	N,N-Dimethylformamide
HOBt	1-Hydroxybenzotriazole
LDA	Lithium bis(trimethylsilyl)amide
NMM	N-Methylmorpholine
THF	Tetrahydrofuran
TFA	Trifluoroacetic acid
TLC	Thin layer chromatography
EDC	N-Ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride

^1H and ^{13}C NMR spectra were recorded using a Bruker AC 250E spectrometer at 250.1 and 62.9 MHz, respectively. Elemental microanalyses were performed by CHN Analysis Ltd., Alpha House, Countesthorpe Road, South Wigston, Leicester LE8 2PJ, UK.

Preparation of Intermediate 1

3R,S-Allyl-2R-isobutyl-1,4-dioic acid-1-pentafluorophenyl-4 *tert*-butyl diester (3:1, RS:RR).

Step1N-(4-Methylpentanoyl)-4S-phenylmethyl-oxazolidin-2-one

A dry 500 ml flask equipped with a magnetic stirrer was charged with 4S-phenylmethyl-oxazolidin-2-one (17.72 g, 100 mmol), this was capped with a rubber septum and flushed with nitrogen. Anhydrous THF (300 ml) was added *via* a cannula and the resulting solution was cooled to -78°C in an acetone/dry-ice bath. A solution of 1.47 M n-butyllithium in hexane (68.4 ml, 101 mmol) was transferred *via* cannula to a dry, septum-stoppered 100 ml dropping funnel. This was added dropwise to the THF solution over 10 minutes.

4-Methylvaleric acid chloride (14.80 g, 110 mmol) was added in one portion by syringe after completion of the addition of n-butyllithium. The resulting solution was stirred at -78°C for 30 minutes and then allowed to warm to ambient temperature over 30 minutes. Excess acid chloride was quenched by the addition of aq. NH₄Cl (60 ml) and the bulk of the solvent was removed under reduced pressure. The resulting slurry was extracted with dichloromethane (2 x 80 ml). The combined

organic extracts were washed with 1M NaOH (75 ml), brine (75 ml), dried (Na_2SO_4 anhyd.) and filtered. The solvent was removed to yield a yellow oil (29.20 g, crude). $^1\text{H-NMR}$; d (CDCl_3), 7.34-7.19 (5H, m), 4.73-4.63 (1H, m), 4.25-4.16 (2H, m), 3.30 (1H, dd, $J=3.3$ Hz), 3.05-2.85 (2H, m), 2.78 (1H, dd, $J=9.5$ Hz), 1.76-1.53 (3H, m) and 0.97 (6H, d, $J=6.2$ Hz).

Step 2

N-(4-(*tert*-Butyl)-2R-isobutyl-butan-1,4-dioyl)-4S-phenylmethyl-oxazolidin-2-one

N-(4-Methylpentanoyl)-4S-phenylmethyl-oxazolidin-2-one (20 g, 72.6 mmol) was placed in a dry 1 litre 3-necked flask to which was added dry THF (400 ml). The mixture was kept under a stream of argon and cooled to -78°C (dry ice/acetone). Sodium bis(trimethyl)silylamide (1M solution in THF, 72.6 ml, 72.6 mmol) was added dropwise through a dropping funnel. After stirring for 20 minutes, *tert*-butyl bromoacetate (21.02 g, 15.8 ml, 109 mmol) was added dropwise over 1 minute, to give an orange solution. The mixture was kept at -78°C and allowed to warm to -50°C over 2 hours. The reaction was then quenched by adding acetic acid (10.90 g, 10.4 ml, 182 mmol) in ether (50 ml) at -50°C , whereupon the solution became colourless. The solvent was removed under reduced pressure and the resulting slurry was partitioned between ethyl acetate and brine. The ethyl acetate layer was washed once with brine and the original brine layer was back-extracted with ethyl acetate. The combined organic layers were dried, filtered and the solvent removed, giving a yellow oil which crystallised on cooling overnight to yield the title compound as a crystalline solid (21.36 g, 76%). $^1\text{H-NMR}$; d (CDCl_3), 7.38-7.24 (5H, m), 4.62-4.72 (1H, m), 4.35-4.20 (1H, m), 4.18-4.16 (2H, m), 3.36 (1H, dd, $J=3.25$ Hz), 2.72 (1H, dd, $J=2.3$ Hz), 2.49 (1H, dd, $J=4.6$ Hz), 1.72-1.24 (3H, m), 1.44 (9H, s) and 0.91-0.96 (6H, dd, $J=4.5$ Hz). $[\alpha]_D^{25} = +66.9^\circ$ ($c=1$, MeOH).

Step 3

2R-Isobutyl-butan-1,4-dioic acid-4-*tert*-butyl ester

N-(4-(*tert*-Butyl)-2R-isobutyl-butan-1,4-dioyl)-4S-phenylmethyl-oxazolidin-2-one (15.30 g, 39 mmol) was placed in a 1 litre flask with a stirrer bar and to it was added a mixture of THF (600 ml) and water (150 ml). The solution was stirred and cooled to 0°C (ice/acetone bath) then 60% aq. H₂O₂ (4.5 ml, 157 mmol) was added *via* syringe over 5 minutes, followed by LiOH (2.65 g, 63 mmol) in 100 ml water. The reaction mixture was stirred for 1h at 0°C. TLC (10% methanol in dichloromethane) showed complete reaction (product gave a yellow spot on TLC on staining with bromocresol green and heating). The reaction mixture was quenched with NaNO₂ (10.88 g, 157 mmol) so that the final pH was 12-13. THF was removed *in-vacuo* and the aqueous layer was extracted with dichloromethane (3 x 200 ml) to recover the chiral auxiliary. The organic extracts were dried (MgSO₄ anhyd.), filtered and the solvent removed *in-vacuo* and the resulting solid chiral auxiliary (7.05 g, 39 mmol, 100%) recrystallised from ethyl acetate-hexane (2:1). [α]_D²⁵ = - 13.0° (c=1, MeOH).

The aqueous layer was cooled in an ice bath and acidified to pH 5-6 with 2M HCl. The resulting cloudy solution was extracted with ethyl acetate (4 x 200 ml), readjusting the pH to 5-6 in between extractions. The combined organic extracts were dried over MgSO₄, filtered and the solvent was removed to yield the title compound as a pale yellow oil (8.21 g, 91%). ¹H-NMR; δd (CDCl₃), 2.85 (1H, m), 2.59 (1H, dd, J=16, 9Hz), 2.38 (1H, dd, J=16, 5Hz), 1.64 (1H, m), 1.28 (1H, m) and 0.93 (6H, dd, J=7, 8Hz). [α]_D²⁵ = + 10.4° (c=1, MeOH).

Step 4

3R,S-Allyl-2R-isobutyl-butan-1,4-dioic acid-4-*tert*-butyl ester (1:9, RS:RR)

To a stirred solution of 2R-isobutyl-1,4-dioic acid-4-*tert*-butyl ester (5 g, 21.7 mmol) in dry THF (100 ml), under an argon atmosphere, at -78°C, was added 1.5M LDA (31.8 ml, 47.74 mmol) dropwise *via* cannula. After stirring the solution at -78°C for 1 hour, allyl bromide (2.44 ml, 28.21 mmol) was added dropwise *via* syringe. The

resulting solution was allowed to warm to room temperature over a 2 hour period. Methanol (10 ml) was added and the solution was stirred at room temperature. After 30 minutes the reaction mixture was concentrated under reduced pressure. The residue was taken up in dichloromethane (100 ml) and washed with 1M HCl (100 ml) and brine (100 ml). The dichloromethane layer was dried over MgSO_4 , filtered and solvent removed under reduced pressure to give the title compound as a golden oil (5.6g, 96.7%) (1:9, RS:RR) $^1\text{H-NMR}$; d (CDCl_3 , major diastereoisomer), 5.78-5.63 (1H, m), 5.01-5.11 (2H, m), 2.57-2.72 (2H, m), 2.37 (2H, m), 1.52-1.67 (2H, m), 1.42 (9H, s), 1.37 (1H, m) and 0.90 (6H, d, $J=6.3$ Hz). $^{13}\text{C-NMR}$; d (CDCl_3 , major diastereoisomer) 181.1, 172.9, 134.6, 117.3, 81.2, 47.8, 44.3, 38.4, 27.9, 25.9, 23.5, and 21.5.

Step 5

3R,S-Allyl-2R-isobutyl-1,4-dioic acid-4-*tert*-butyl ester (3:1, RS:RR)

(i) To a stirred solution of 3R,S-Allyl-2R-isobutyl-1,4-dioic acid-4-*tert*-butyl ester (1:9, RS:RR) (5.11 g, 18.9 mmol) in dry THF (100 ml) under argon at -78°C was added 1.5M LDA (27.7 ml, 41.6 mmol) *via* cannula. The reaction mixture was warmed to room temperature over a 2 hour period then cooled back to -78°C and methanol (8 ml) was added *via* syringe. The reaction was then allowed to warm to room temperature for a further 2 hours. The solvent was removed under reduced pressure. The residue was taken up in dichloromethane (150 ml) and washed with 1M HCl (150 ml) and brine (150 ml). The dichloromethane layer was dried over MgSO_4 , filtered and the solvent removed under reduced pressure to yield the title compound (3:2, RS:RR), as a brown oil (4.7 g, 92%).

(ii) Utilising the epimerisation procedure described in Example 3b(i), but employing a reaction temperature of -78°C after addition of LDA *in lieu* of allowing the reaction mixture to warm to room temperature yielded the title compound as a brown oil (4.6 g, 98%) (3:1, RS:SR). $^1\text{H-NMR}$; d (CDCl_3 , major diastereoisomer),

11.60 (1H, br s), 5.75-5.61 (1H, br m), 5.06-4.96 (2H, br m), 2.70-2.52 (2H, br m), 2.36-2.19 (2H, br m), 1.65-1.44 (2H, br m), 1.40 (9H, s), 1.13 (1H, m) and 0.86 (6H, dd, $J=4.4, 2.1$ Hz). ^{13}C -NMR; d (CDCl_3 , major diastereoisomer) 180.7, 172.2, 134.6, 117.1, 81.0, 48.6, 45.7, 38.9, 34.8, 33.4, 27.9, 26.2 and 21.2.

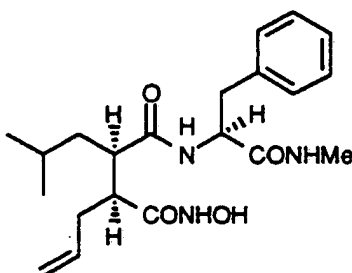
Step 6

3R,S-Allyl-2R-isobutyl-1,4-dioic acid-1-pentafluorophenyl-4 *tert*-butyl diester (3:1, RS:RR)

To a stirred solution of 3R,S-Allyl-2R-isobutyl-1,4-dioic acid-4 *tert*-butyl ester (4.60 g, 17.2 mmol) (3:1, RS:RR) in dichloromethane (50 ml) was added pentafluorophenol (6.13g, 33.3 mmol). The reaction mixture was cooled to 0°C and NMM (2.02 g, 20.0 mmol) and EDC (3.94 g, 20.0 mmol) were added. The reaction mixture was allowed to warm to room temperature and stirred for 12 hours then the solvent was removed under reduced pressure. The residue was taken up in dichloromethane (50 ml) and washed with 1M HCl (3 x 50 ml), saturated sodium bicarbonate (3 x 50 ml) and brine (50 ml). The dichloromethane layer was dried over MgSO_4 , filtered and the solvent removed under reduced pressure to give a brown oil. Column chromatography (flash silica, dichloromethane) yielded the title compound as a golden oil (5.47 g, 74%) (3:1, RS:SR). ^1H -NMR; d (CDCl_3 , major diastereoisomer), 5.85-5.67 (1H, br m), 5.17-5.05 (2H, br m), 3.10-3.01 (1H, m), 2.79-2.69 (1H, m), 2.51-2.29 (2H, br m), 1.88-1.61 (2H, br m), 1.46 (9H, s), 1.37-1.24 (1H, m) and 0.96 (6H, dd, $J=4.0, 4.5$ Hz). ^{13}C -NMR; d (CDCl_3 , major diastereoisomer), 171.5, 170.3, 134.1, 117.5, 81.4, 48.8, 45.8, 39.5, 35.0, 27.9, 26.3, 23.5, and 21.0.

Example 1

3R-(2-Phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-2S-2-propenyl-hexanohydroxamic acid



Example 1a

3R,S-(2-Phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-2S-2-propenyl-hexanoic acid-4-*tert*-butyl ester

A solution of 3R,S-allyl-2R-isobutyl-succinic acid 4-tert-butyl 1-pentafluorophenyl diester (7.0 g, 16.0 mmol) (3:1, RS:RR) and S-phenylalanine methylamide (3.72 g, 20.9 mmol) in DMF (70 ml) was stirred at 45°C for 8 hours and at room temperature for 24 hours. The DMF was removed under reduced pressure. The residue was taken up in dichloromethane (50 ml) and washed with sodium carbonate (1M, 2 x 150 ml), 1M HCl (2 x 150 ml) and brine (150 ml). The dichloromethane layer was dried over MgSO₄, filtered and the solvent was removed under reduced pressure to give a golden oil. Column chromatography (flash silica; 1:1 ethyl acetate: hexane) yielded the title compound as a white solid after recrystallisation from ethyl acetate/hexane (10:1 diastereomer ratio, 4.05g, 59%). ¹H-NMR; δ (CDCl₃, major diastereoisomer), 7.29 to 7.21 (5H, m), 6.35 (1H, d, J=7.6 Hz), 5.94 (1H, br s), 5.69

to 5.52 (1H, br m), 4.98 (1H, s), 4.93 (1H, d, J=7.7 Hz), 4.64 (1H, d, J=7.4 Hz), 3.07 (2H, d, J=7.4 Hz), 2.72 (3H, d, J=1.7 Hz), 2.52 to 2.30 (2H, br m), 2.05 to 1.80 (2H, br m), 1.62 (1H, m), 1.43 (10H, s and m), 1.87 (1H, m), and 0.84 (6H, dd, J=3.6, 2.9 Hz).

Example 1b

3R-(2-Phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-2S-2-propenyl-hexanoic acid

To a solution of 3R,S-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-2S-2-propenyl-hexanoic acid-4-*tert*-butyl ester (4.05 g, 9.4 mmol) (10:1, SRS:RRS) in dichloromethane (25 ml) at 0°C was added TFA (12 ml). The reaction flask was placed in the fridge for 12 hours. Excess TFA and solvent were removed under reduced pressure. The residue was taken up in dichloromethane (20 ml), and washed with brine (2 x 25 ml). The dichloromethane layer was dried over MgSO₄ and solvent removed under reduced pressure to give a white solid, which was recrystallised from ethyl acetate/hexane to yield the title compound as a white crystalline solid (3.3 g, 94%).

¹H-NMR; δ (CD₃OD), 8.41 (1H, d, J=7.9 Hz), 7.83 (1H, br s), 7.23 to 7.11 (5H, br m), 5.58 to 5.42 (1H, br m), 4.88 (2H, m), 4.63 (1H, m), 3.03 (1H, dd, J=8.1, 5.6 Hz), 2.84 (1H, dd, J=9.9, 3.7 Hz), 2.65 (3H, d, J=4.5 Hz), 2.48 (1H, dt, J=7.7, 2.9 Hz), 2.31 (1H, dt, J=6.8, 3.7 Hz), 1.85 (1H, m), 1.52 (2H, m), 1.30 (1H, br m), 1.00 (1H, m), and 0.81 (6H, dd, J=10.3, 6.4 Hz).

Example 1c

3R-(2-Phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-2S-2-propenyl-hexanohydroxamic acid

To a solution of 3R-(2-phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-2S-2-propenyl-hexanoic acid (2.52 g, 6.73 mmol) in DMF (50 ml) was added HOBt (1.18g, 8.75 mmol), NMM (0.88 g, 8.75 mmol) and EDC(1.68g, 8.75 mmol). The

reaction mixture was cooled to 0°C and hydroxylamine hydrochloride (0.94 g, 13.5 mmol), NMM (1.36 g, 13.5 mmol) were added. The reaction mixture was allowed to warm to room temperature and stirred for 12 hours. Removal of the solvent under reduced pressure left an oil to which was added an ether water mixture (1:1, 80 ml). The precipitated solid was collected by filtration then recrystallisation from methanol/diisopropyl ether yielded the title compound as a white solid (single diastereoisomer, 1.97g, 75%).

m.p. 235 - 236 °C

Analysis calculated for $C_{21}H_{31}N_3O_4 \cdot 0.5H_2O$

Requires C 63.29, H 8.09, N 10.54

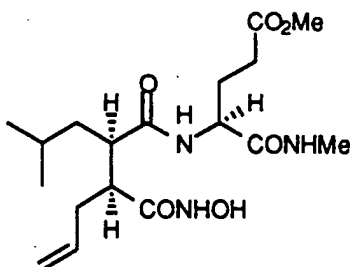
Found C 63.16, H 8.02, N 10.73

1H -NMR; δ (CD_3OD), 8.51 (1H, d, $J=8.0$ Hz), 7.85 (1H, br s), 7.26 - 7.10 (5H, br m), 5.50 - 5.35 (1H, br m), 4.82 (2H, m), 4.64 (1H, dd, $J=9.2, 5.5$ Hz), 3.03 (1H, dd, $J=8.4, 5.3$ Hz), 2.84 (1H, dd, $J=10.0, 3.6$ Hz), 2.66 (3H, d, $J=3.3$ Hz), 2.42 (1H, m), 2.02 - 1.78 (2H, br m), 1.44 (3H, br m), 1.17 - 0.93 (1H, br m), and 0.81 (6H, dd, $J=10.6, 6.4$ Hz).

^{13}C -NMR; δ (CD_3OD) 176.2, 173.9, 172.4, 138.5, 136.1, 130.3, 129.4, 127.9, 117.2, 56.0, 41.6, 38.9, 35.7, 26.9, 26.2, 24.4, and 21.7.

Example 2

3R-(3-Methoxycarbonyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-2S-2-propenyl-hexano hydroxamic acid



Prepared by methods analogous to those described in Example 1, starting from S-5-methylglutamic acid N-methylamide.

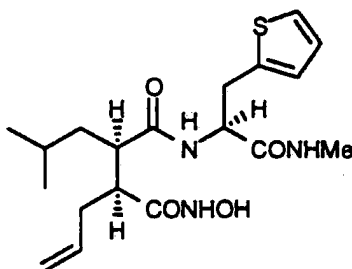
m.p. 228 - 229 °C

¹H-NMR; δ (CD₃OD), 5.63 (1H, br m), 4.97 (2H, m), 4.33 (1H, m), 3.62 (3H, s), 2.68 (3H, s), 2.53 (1H, m), 2.38 (2H, t, J=7.2 Hz), 2.20 (2H, m), 2.11 - 1.91 (3H, br m), 1.56 - 1.45 (2H, br m), 1.02 (1H, m) and 0.82 (6H, dd, J=7.3, 6.2 Hz).

¹³C-NMR; δ (CD₃OD), 177.2, 174.8, 173.8, 172.3, 136.0, 117.5, 53.7, 52.2, 41.6, 36.2, 31.0, 28.0, 26.9, 26.2, and 21.7.

Example 3

3R-(1S-Methylcarbamoyl-2-thien-2-yl-ethylcarbamoyl)-5-methyl-2S-2-propenyl-hexanohydroxamic acid



Prepared by methods analogous to those described in Example 1, starting from S-2-thienylalanine N-methylamide.

m.p. 216 - 217 °C

Analysis calculated for $C_{19}H_{29}N_3O_4S \cdot 0.3H_2O$

Requires C 56.92, H 7.44, N 10.48, S 8.00 %

Found C 57.01, H 7.26, N 10.23, S 8.06 %

1H -NMR; δ (CD_3OD), 7.11 (1H, dd, $J=1.0, 4.0Hz$), 6.87 - 6.79 (2H, m), 5.54 - 5.39 (1H, m), 4.80 (2H, m), 4.60 (1H, dd, $J=6.0, 3.1Hz$), 3.43 - 3.06 (3H, m), 2.64 (3H, s), 2.46 (1H, m), 1.99 (2H, m), 1.56 (1H, br m), 1.32 (1H, br m), 0.97 (1H, br m), 0.82 (3H, d, $J=6.4Hz$), and 0.75 (3H, d, $J=6.4Hz$).

^{13}C -NMR; δ (CD_3OD) 176.4, 173.3, 172.3, 140.3, 136.8, 127.8, 127.6, 125.2, 117.2, 56.2, 41.6, 35.7, 32.9, 27.0, 26.2, 24.2, and 21.8.

CC(C)C[C@H](C(=O)N[C@@H](C)C(=O)N)C[C@H](C)C=C

m.p. 226 - 228 °C

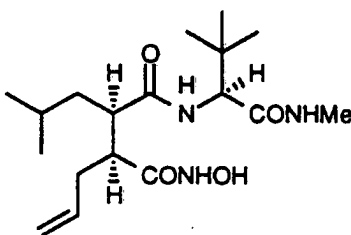
Requires C 60.51, H 9.37, N 11.76 %

Found C 60.48, H 9.21, N 11.67 %

¹³C-NMR; δ ((CD₃)₂SO, 333K), 173.3, 172.2, 169.4, 136.0, 115.8, 51.1, 46.2, 45.9, 34.7, 25.4, 25.3, 24.3, 23.9, 23.0, 21.6, and 21.4.

Example 5

3R-(2,2-Dimethyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-2S-2-propenyl-hexanohydroxamic acid



Prepared by methods analogous to those described in Example 1, starting from *S*-*tert*-leucine N-methylamide.

m.p. 229.5 - 231.5 °C

Analysis calculated for $C_{18}H_{33}N_3O_4 \cdot 0.2H_2O$

Requires: C 60.21, H 9.38, N 11.70 %

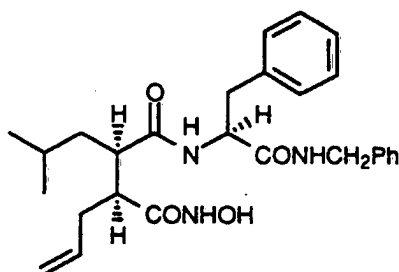
Found: C 60.34, H 9.23, N 11.61 %

1H -NMR; δ (CD_3OD), 8.05 (1H, d, $J = 9.0$ Hz), 7.96 (1H, br s), 5.55 - 5.71 (1H, br m), 4.96 (2H, m), 4.23 (1H, d, $J = 9.0$ Hz), 2.66 (4H, m and d, $J = 4.6$ Hz), 2.03 - 2.33 (3H, br m), 1.48 (1H, m), 1.34 (1H, m), 0.98 (10H, s and m), 0.84 (3H, d, $J = 6.4$ Hz) and 0.79 (3H, d, $J = 6.5$ Hz).

^{13}C -NMR; δ (CD_3OD), 176.5, 173.0, 172.4, 136.1, 117.4, 62.5, 41.8, 36.4, 35.1, 27.3, 27.1, 26.0, 24.3 and 22.0.

Example 7

3R-(2-Phenyl-1S-phenylmethylcarbamoyl-ethylcarbamoyl)-5-methyl-2S-2-propenyl-hexanohydroxamic acid



Prepared by methods analogous to those described in Example 1, starting from S-phenylalanine N-benzylamide.

m.p. 236 - 237°C

¹H-NMR; d ((CD₃)₂SO), 8.73 (1H, d, J=1.4Hz), 8.33 (2H, m), 7.22 (10H, m), 5.34 (1H, m), 4.81-4.65 (3H, m), 4.28 (2H, dd, J=6.2, 12.4Hz), 2.99 (1H, dd, J=4.5, 13.7Hz), 2.85 (1H, dd, J=4.0, 8.0Hz), 2.50-2.48 (1H, m), 1.93-1.79 (2H, m), 1.35-1.25 (3H, m), 0.89-0.69 (1H, m), and 0.89-0.69 (6H, dd, J=6.4, 6.0Hz).

¹³C-NMR; d ((CD₃)₂SO), 173.3, 171.1, 169.3, 139.1, 137.9, 135.9, 129.1, 128.1, 127.9, 127.2, 126.7, 126.2, 115.6, 54.1, 46.2, 45.8, 42.3, 37.5, 34.2, 25.2, 23.9 and 21.5.

Biological Example A

The potency of compounds of the invention as inhibitors of collagenase was determined by the procedure of Cawston and Barrett, (Anal. Biochem., 99, 340-345, 1979), hereby incorporated by reference, whereby a 1 mM solution of the compound being tested, or a dilution thereof, was incubated at 37° for 16 hours with collagen and collagenase (buffered with 25mM Hepes, pH 7.5 containing 5mM CaCl₂, 0.05% Brij 35 and 0.02% NaN₃). The collagen was acetylated ¹⁴C collagen prepared by the method of Cawston and Murphy, (Methods in Enzymology, 80, 711, 1981), hereby incorporated by reference. The samples were centrifuged to sediment undigested collagen, and an aliquot of the radioactive supernatant removed for assay on a scintillation counter as a measure of hydrolysis. The collagenase activity in the presence of 1 mM of the test compound, or a dilution thereof, was compared to activity in a control devoid of inhibitor and the result reported below as that of inhibitor concentration effecting 50% inhibition of the collagenase activity (IC₅₀).

The potency of compounds of the invention as inhibitors of stromelysin was determined by the procedure of Cawston et al, (Biochem. J., 195, 159-165, 1981), hereby incorporated by reference, whereby a 1 mM solution of the compound being tested, or a dilution thereof, was incubated at 37° for 16 hours with stromelysin and ¹⁴C acetate casein (buffered with 25mM Hepes, pH 7.5 containing 5mM CaCl₂, 0.05% Brij 35 and 0.02% NaN₃). The casein was acetylated ¹⁴C casein prepared by the method of Cawston et al (ibid). The stromelysin activity in the presence of 1 mM of the test compound, or a dilution thereof, was compared to activity in a control devoid of inhibitor and the result reported below as that of inhibitor concentration effecting 50% inhibition of the stromelysin activity (IC₅₀).

Results:

Compound	Collagenase IC ₅₀ (nM)	Stromelysin IC ₅₀ (nM)
Example 1	10	30
Example 5	3-4	80

Example 7

10

5

Biological Example B

The biological efficacy of a compound of the invention in chronic disease states is a function of the systemic blood concentration maintained over time, and its intrinsic potency. These factors can be quantified as $IC_{50} \cdot hrs$ (24hrs), which parameter is defined as the total number of IC_{50} s in the blood over the 24 hours post dosing, the IC_{50} being defined as the concentration of the compound that reduces collagenase activity to 50% of its normal value.:

Test compounds were administered at 10 mg/kg in PBS/0.1% Tween 80 by gavage to 6 male rats (300g) per treatment group. Blood samples were removed by tail venepuncture at 0.5, 1.0, 2.0, 6.0 and 24 hours post administration. 0.5 ml of blood was placed into 4.5ml tubes containing 0.1ml acid citrate dextrose (ACD) as anti-coagulant. For extraction, 3ml methanol was added and the precipitated blood pelleted by centrifugation (30 min at 3000 rpm). A 2ml aliquot of supernatant was removed and dried by lyophilisation. The extract was redissolved in 200 μ l DMSO and a 10 μ l aliquot assayed for collagenase inhibitory activity. The inhibitory activity in the extracts was determined using the collagenase assay described in Biological Example A above, and the concentration of inhibitor (that is drug plus any active metabolites) obtained by comparison with standard curves. Results are expressed as area under the curve (AUC) $IC_{50} \cdot hrs$ (24hrs), the IC_{50} being defined as the concentration of the compound that reduces collagenase activity to 50% of its normal value.

Results

Compound	AUC ($IC_{50} \cdot hrs$ (24hrs))
Example 1	233
Example 5	970

Biological Example C

The compound of Example 1 was tested in a rat adjuvant arthritis model.

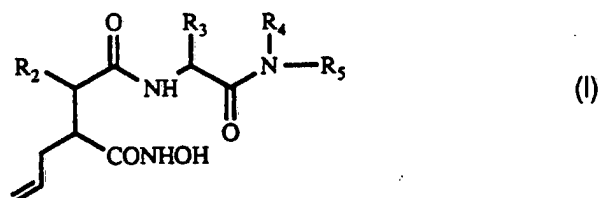
Adjuvant arthritis was produced in male Lewis rats (Charles River) by a single intradermal injection of 0.75mg of M. butyricum in light parafin oil (Freund's complete adjuvant) into the base of the tail. The "secondary" lesion occurs after a delay of 10 days, and is characterised by inflammation of the hindpaws. Hindpaw oedema volumes were measured plethysmographically by water displacement. Animals in the treated groups received two doses of 10 mg/kg of the test compound in PBS/0.1% Tween orally, each day, from day 13 to day 17. Results are reported as paw volume change from the volume measured on day 13.

Results:

Compound of	Day 17	Day 20
Example 1	(% relative to day 13)	(% relative to day 13)
	52%	65%

Claims:

1. A compound of formula (I) :



wherein

R_2 represents a C_2 - C_6 alkyl group which may contain an ether or thioether linkage;

R_3 represents (a) the side chain of a naturally occurring alpha-amino acid in which any carboxylic acid groups may be esterified or amidated, any hydroxyl or thiol groups may be acylated or alkylated (etherified) and any amino groups may be acylated, or (b) a group $R_6(A)_n$ wherein n is 0 or 1, A represents a divalent C_1 - C_6 alkyl or C_2 - C_6 alkenyl group optionally interrupted by one or more -O-, or -S- atoms or -N(R_7)- groups where R_7 is hydrogen or C_1 - C_6 alkyl, and R_6 is a phenyl or heterocyclyl group either of which may be substituted, or (except where n is 0) a hydrogen atom;

R_4 represents hydrogen or methyl;

R_5 represents hydrogen, C_1 - C_6 alkyl or phenyl(C_1 - C_6 alkyl),

or a salt, solvate or hydrate thereof.

2. A compound as claimed in claim 1 wherein the stereochemistry is as follows:

C atom carrying the allyl group and hydroxamic acid moiety - S,

C atom carrying the R_2 group - R,

C atom carrying the R_3 group - S.

3. A compound as claimed in claim 1 or claim 2 wherein R_2 represents an

isobutyl group.

3. A compound as claimed in any one of the preceding claims wherein R_3 represents a phenylmethyl (benzyl) group in which the phenyl ring is optionally substituted with a C_1 - C_4 alkoxy or $HOOC$ -(C_1 - C_4 alkyl)-O- group, a thienylmethyl group, a C_1 - C_6 alkyl group, or a CH_2CO_2 (C_1 - C_4)alkyl or $CH_2CH_2CO_2$ (C_1 - C_4)alkyl group.

4. A compound as claimed in claim 3 wherein R_3 represents a tertbutyl group.

5. A compound as claimed in any one of the preceding claims wherein R_4 represents a hydrogen atom.;

6. A compound as claimed in any one of the preceding claims wherein R_5 represents a (C_1 - C_4)alkyl group or a benzyl group.

7. A compound selected from the group consisting of

3R-(3-methoxycarbonyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-2S-2-propenyl-hexanohydroxamic acid;

3R-(1S-methylcarbamoyl-2-thien-2-yl-ethylcarbamoyl)-5-methyl-2S-2-propenyl-hexanohydroxamic acid;

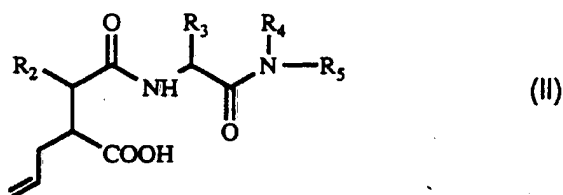
3R-(3-Methyl-1S-methylcarbamoyl-butylcarbamoyl)-5-methyl-2S-2-propenyl-hexanohydroxamic acid;

2S-[1S-methylcarbamoyl-2-oxadiazol-5-yl-ethylcarbamoyl)-5-methyl-2S-2-propenyl-hexanohydroxamic acid;

and salts, solvates or hydrates thereof.

8. 3R-(2-Phenyl-1S-methylcarbamoyl-ethylcarbamoyl)-5-methyl-2S-2-propenyl-hexanohydroxamic acid and its salts, solvates or hydrates.

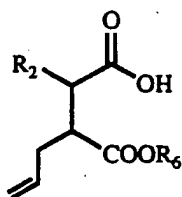
9. 3R-(2,2-Dimethyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-2S-2-propenyl-hexanohydroxamic acid and its salts, solvates or hydrates.
10. 3R-(2-Phenyl-1S-phenylmethylcarbamoyl-ethylcarbamoyl)-5-methyl-2S-2-propenyl-hexanohydroxamic acid and its salts, solvates or hydrates.
11. A process for the preparation of a compound as claimed in claim 1 comprising:
 - (a) coupling an acid of general formula (II)



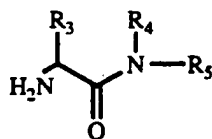
or an activated derivative thereof with hydroxylamine, O-protected hydroxylamine, or a salt thereof, R_2 , R_3 , R_4 , and R_5 being as defined in claim 1 in relation to general formula (I) except that any substituents in R_2 , R_3 , R_4 , and R_5 which are potentially reactive with hydroxylamine, O-protected hydroxylamine or their salts may themselves be protected from such reaction, then removing any protecting groups from the resultant hydroxamic acid moiety and from any protected substituents in R_2 , R_3 , R_4 , and R_5 ; and

- (b) optionally converting a compound of general formula (I) into another compound of general formula (I).
12. A process as claimed in claim 11 wherein an activated derivative of a compound of formula (II) is used and said activated derivative is a pentafluorophenyl, hydroxysuccinyl, or hydroxybenztriazyl ester.
13. A process as claimed in claim 11 or claim 12 wherein the compound of

general formula (II) is prepared by coupling an acid of formula (III) or an activated derivative thereof with an amine of formula (IV)



(III)



(IV)

wherein R_2 , R_3 , R_4 , and R_5 are as defined in claim 1 in relation to general formula (I) and R_6 represents C_1 - C_6 alkyl, or benzyl.

14. A method of management (by which is meant treatment or prophylaxis) of diseases or conditions mediated by MMPs and/or TNF in mammals including humans, which method comprises administering to the mammal an effective amount of a compound as claimed in any one of claims 1 to 10.

15. A compound as claimed in any one of claims 1 to 10 for use in human or veterinary medicine, particularly in the management (by which is meant treatment or prophylaxis) of diseases or conditions mediated by MMPs and/or TNF.

16. A compound as claimed in any one of claims 1 to 10 for use in human or veterinary medicine in the management (by which is meant treatment or prophylaxis) of diseases or conditions mediated by MMPs and/or TNF.

17. The use of a compound as claimed in any one of claims 1 to 10 in the preparation of an agent for the management (by which is meant treatment or prophylaxis) of diseases or conditions mediated by MMPs and/or TNF.

18. A method as claimed in claim 14, a compound for use as claimed in claim 15 or claim 16, or the use as claimed in claim 17, wherein the diseases or condition referred to is one mediated by an MMP.

19. A method as claimed in claim 14, a compound for use as claimed in claim 15 or claim 16, or the use as claimed in claim 17, wherein the diseases or condition referred to is rheumatoid arthritis, osteoarthritis, periodontitis, gingivitis, corneal ulceration or tumour invasion by secondary metastases.

20. A method as claimed in claim 14, a compound for use as claimed in claim 15 or claim 16, or the use as claimed in claim 17, wherein the diseases or condition referred to is one mediated by TNF.

21. A method as claimed in claim 14, a compound for use as claimed in claim 15 or claim 16, or the use as claimed in claim 17, wherein the disease or condition referred to is inflammation, fever, cardiovascular effects, haemorrhage, coagulation and acute phase response, cachexia and anorexia, an acute infection, a shock state, a graft versus host reaction or autoimmune disease.

22. A pharmaceutical or veterinary composition comprising a compound as claimed in any of claims 1 to 10 together with a pharmaceutically or veterinarily acceptable excipient or carrier.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 94/00495

A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 C07D333/24 C07D271/06 C07C259/06 A61K31/38 A61K31/42
A61K31/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C07D C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 489 579 (CELLTECH LIMITED) 10 June 1992 cited in the application see abstract see page 3, line 18 - line 26 ---	1,11, 14-22
X	WO,A,91 02716 (BRITISH BIO-TECHNOLOGY LIMITED) 7 March 1991 cited in the application see abstract; claims see page 9, line 24 - line 30 ---	1,11, 14-22
X	EP,A,0 489 577 (CELLTECH LIMITED) 10 June 1992 cited in the application see abstract; claims see page 3, line 22 - line 26 ---	1,11, 14-22
	-/--	



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

26 April 1994

Date of mailing of the international search report

- 3. 05. 94

Name and mailing address of the ISA

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Paisdor, B

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 94/00495

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>EP,A,0 236 872 (F. HOFFMANN-LA ROCHE & CO. AKTIENGESELLSCHAFT) 16 September 1987 cited in the application see page 22, line 56; claim 1 -----</p>	1-22

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB94/00495

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 14-22 are directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 94/00495

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0489579	10-06-92	AU-A- 9017391	25-06-92
		AU-A- 9023391	25-06-92
		EP-A- 0489577	10-06-92
		WO-A- 9209564	11-06-92
		WO-A- 9209565	11-06-92
		GB-A- 2255339	04-11-92
		GB-A- 2255340	04-11-92
		HU-A- 61973	29-03-93
		JP-T- 5503719	17-06-93
		JP-T- 5503720	17-06-93
		US-A- 5300501	05-04-94
WO-A-9102716	07-03-91	AU-B- 639706	05-08-93
		AU-A- 6045490	03-04-91
		EP-A- 0489032	10-06-92
		JP-T- 5501864	08-04-93
EP-A-0489577	10-06-92	AU-A- 9017391	25-06-92
		AU-A- 9023391	25-06-92
		EP-A- 0489579	10-06-92
		WO-A- 9209564	11-06-92
		WO-A- 9209565	11-06-92
		GB-A- 2255339	04-11-92
		GB-A- 2255340	04-11-92
		HU-A- 61973	29-03-93
		JP-T- 5503719	17-06-93
		JP-T- 5503720	17-06-93
		US-A- 5300501	05-04-94
EP-A-0236872	16-09-87	AU-B- 588437	14-09-89
		AU-A- 6990287	17-09-87
		CA-A- 1314655	16-03-93
		DE-A- 3782751	07-01-93
		JP-A- 62230757	09-10-87
		US-A- 4996358	26-02-91